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APPLICATIONS OF BIOLOGICAL RESEARCH IN THE CONTROL OF INDUSTRIAL WASTES

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Environmental Health Center
Cincinnati, Ohio

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By

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APPLICATION OF BIOLOGICAL RESEARCH IN THE CONTROL OF INDUSTRIAL WASTES

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At the Environmental Health Center a variety of approaches is being used in the attack on the complex problems which arise from the discharge of industrial wastes into the waters of our nation. The Biology Section is participating in these studies and is directing its investigations toward the following objectives: 1. The development of a simple biological method which can be used in most streams throughout the year for indicating the severity and extent of pollution and the degree of recovery; 2. A summarization of present knowledge on the toxic effects of industrial wastes to fish life and the pointing out of gaps in our knowledge of this subject through a literature survey; 3. The devising and standardizing of bioassay procedures for determining the toxicity of industrial wastes to fishes; and 4. The ascertaining of the toxicity of certain wastes to fishes by means of bioassays.

In the biological approach to the industrial waste problem we are concerned with the effects of the wastes on aquatic life. It is desirable, therefore, to classify pollutants according to these effects on the aquatic environment and life and to set up some measure or standard for suitable water quality.

Functional Classification of Pollutants

The usual procedure is to classify aquatic pollutants according to their source, as domestic sewage and industrial wastes. From the viewpoint of the aquatic biologist, a functional classification of pollutants based on their effects

on receiving waters and the resident aquatic life is more desirable. With such a classification all pollutants may be roughly grouped under five headings as follows: (1) inert organic material; (2) Putrescible wastes; (3) toxic wastes; (4) radioactive wastes; and (5) wastes of significant heat content. Some materials will fall under more than one heading, for a waste may be putrescible and also exhibit toxic properties at some stage of its decomposition.

Inert inorganic materials include silt, sand, clay, waste from hydraulic and placer mining, mine washings, and wastes from ore refineries or certain manufacturing processes. Their general effects on streams are to fill pools, smother rich, food-producing riffles, cause excessive turbidity or, in extreme instances, completely fill the stream channel and convert the stream into a shallow meandering sheet of water largely devoid of food organisms. Among pollutants of this type, materials from land erosion are of outstanding importance. Although other types of wastes are of great importance, especially in industrial areas, the products of erosion, are, perhaps our most damaging pollutant. Under our present system of land use, few streams are free of turbidity and coarser materials from land erosion. Although these materials have limited direct effect on fishes they drastically curtail food production and destroy pools such that the fish yield is decreased. Their universal occurrence is what makes this class of pollutants so damaging.

Putrescible wastes may adversely affect aquatic life in a number of ways. The most important effect is the depletion of the dissolved oxygen by the bacteria which attack the organic wastes and break them down into simpler

substances. Further, large amounts of some organic materials promote dense growths of Sphaerotilus, protozoa, and zoogloea which cover the stream bottom smothering the large bottom organisms and eliminating the principal food of fishes. When dissolved oxygen is depleted the anaerobic decomposition of organic wastes may result in the production of toxic materials such as H_2S. Putrescible wastes are discharged into our waters from a wide variety of sources such as domestic sewage, wastes from canneries, tanneries, sugar refineries, milk plants, cheese factories, breweries, paper mills, etc.

Toxic wastes may cause direct poisonous effects when they enter the bodies of fishes, or they may exert external harmful effects such as the coagulation of the mucus of the gills by acids or certain metallic ions such as copper, which leads to smothering. A variety of ions and compounds are toxic to fish in even relatively small quantities; among these are copper, zinc, lead, phenol, and cyanide.

At present little is known concerning the effects of radioactive wastes on aquatic life or their possible danger to humans who may use the aquatic organisms as food. There is some concentration of certain radioactive materials in fishes and as atomic energy is adapted to industrial uses, there will be biological problems connected with the disposal of radioactive wastes.

Hot wastes, such as cooling waters, can raise stream temperatures so they are unfavorable for most aquatic life. In trout and salmon streams even slight rises in stream temperatures may be undesirable.

Definition of suitable aquatic conditions or water quality standards

Many water quality standards have been proposed in the past, and some

have been adopted by several states. Those who have had experience with them, however, have found that the proposed standards have rather limited application for the protection of wildlife or are satisfactory in only limited areas. A set of standards simply listing the allowable amounts of acids, alkalies, cyanide, lead, copper, zinc, and other materials which can be safely discharged into our streams would be very useful. Under our present state of knowledge, however, a chemical definition of suitable environmental conditions applicable to all waters and fish in general cannot be given.

Because of the diverse requirements of the different species of fishes, the diversity in the character of receiving waters, the variability of wastes and their antagonistic and synergistic action, it is best to judge the suitability of a water for fish life by its fish yield in comparison with other similar waters which are free or relatively free from pollution. The fish crop represents the end product of all the biological processes in the aquatic habitat and it reflects over-all biological conditions in the water. If a satisfactory sustained yield is produced, conditions may be considered satisfactory. If environmental conditions are unfavorable they will be indicated by a poor fish yield.

If our aim is to preserve sport and commercial fishing, the measure of success is the sustained annual yield or crop of the desired species of fish. In abating pollution, however, we cannot expect streams to be returned to their pre-colonial purity. Erosion, silting, and agricultural use of land have made that an impossibility. Further, some use must be made of our streams for the disposal of wastes as it is economically not feasible to do otherwise. Some wastes may create conditions which are unfavorable for certain inverte-

brates and limit the production of certain fish food organisms. However, if they are replaced by other organisms such that the yield of the game and commercial fishes is not adversely affected, conditions should be considered as satisfactory for fish life. In summary, the determination of water suitability for fish life should be based on fish productivity as determined by the fish yield in pounds per acre, the catch per man hour, the quality of the fish flesh, species composition, and standing population as compared to average conditions of the area.

Stream Sanitation Studies

For two and one-half years continuous studies have been carried out on Lytle Creek, a small stream which lies some 45 miles northeast of Cincinnati, and receives the discharge of the primary treatment plant of Wilmington, Ohio. The aim of these field studies is to develop a biological method which can be used on most streams at all seasons of the year to indicate the severity and extent of pollution and the degree of recovery.

In meeting this objective it is essential to relate aquatic populations to the environmental conditions under which they develop. Ecological conditions prevailing during the entire annual cycle are of importance and must be studied. Monthly samplings and weekly observations have been made on Lytle Creek to determine changes in environmental conditions with the progression of the seasons and the effects of these changes on aquatic life. In addition, hourly samples have been taken over 24 hour periods at 8 representative stations along the stream two or more times each season to determine nocturnal and diurnal fluctuations in important environmental conditions. A regular annual

cycle of stream conditions has been found. During the winter months there was an abundance of dissolved oxygen throughout the length of the stream. As the air and water temperatures increased in the spring and before the leaves were on the trees, oxygen, due to the photosynthetic action of the algae, began to appear in high concentrations in the afternoon, particularly in the upper half of the recovery zone. As the water became warmer, oxygen began to disappear between 2 and 6 A. M. in the section of stream immediately below the outfall of the sewage treatment plant. This oxygen deficiency became progressively greater in extent and duration as spring changed to summer and during summer, there was a 2 mile septic zone below the outfall. At night the oxygenless zone extended one to two miles farther downstream and there were extensive nocturnal variations in dissolved oxygen in the recovery zone. As the fall season advanced the process was reversed. The oxygenless zone became progressively shorter in extent and duration until in winter there was abundant oxygen throughout the stream at all times.

The variations in dissolved oxygen during a 24 hour cycle are illustrated by figures 1 through 3. In these and succeeding figures, sampling stations are designated as distance in miles above the stream mouth. Station 8.7 is in the upper clean water zone; station 7.6 is in a zone of partial pollution due to street drainage; the outfall of the sewage treatment plant is at station 7.2; Station 6.5 is in the septic zone of summer; stations 5.2 and 4.2 are in the recovery zone; station 2.8 is in the lower recovery zone or the upper clean water area; and station 1.0 is in the clean water zone. It will be noted that oxygen was lowest in the septic zone and highest in the recovery zone.

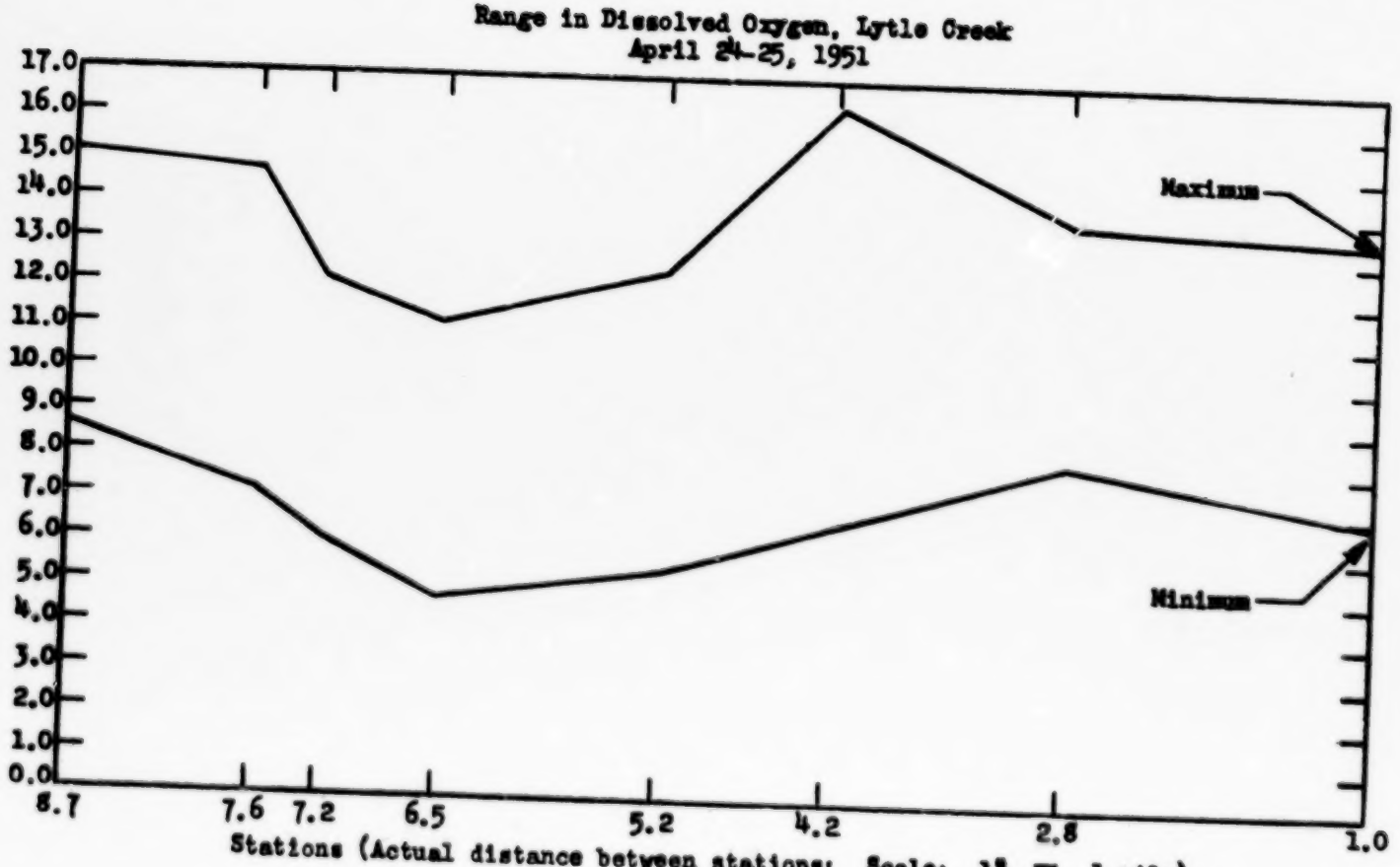

Range in Dissolved Oxygen, Lytle Creek
April 24-25, 1951
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Maximum
Minimum
8.7
7.6
7.2
6.5
5.2
4.2
2.8
1.0
Stations (Actual distance between stations: Scale: 1" = 1 mile)

Figure 1

7-a

Range in Dissolved Oxygen, Lytle Creek
August 22-23, 1951

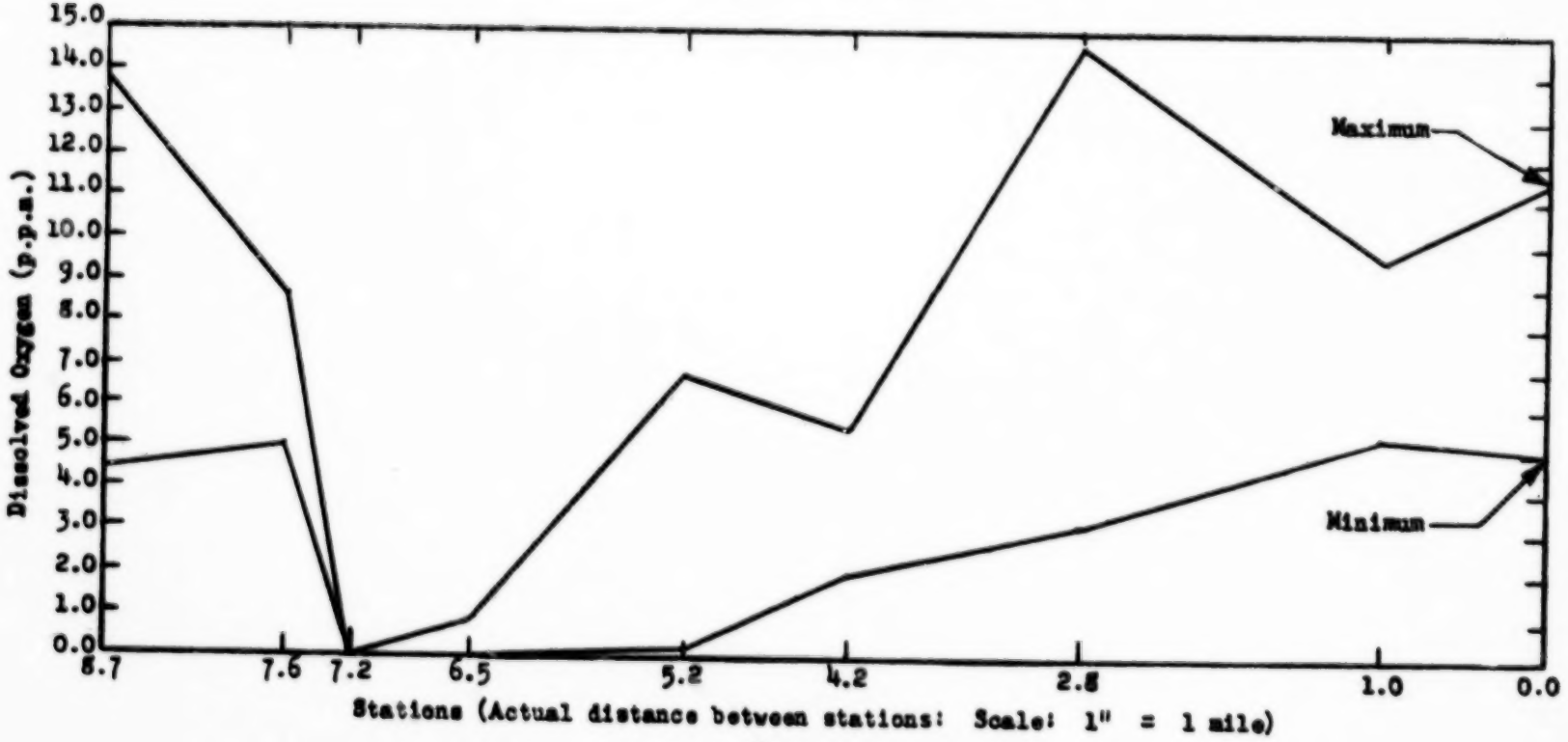


Figure 2

Range in Dissolved Oxygen, Lytle Creek

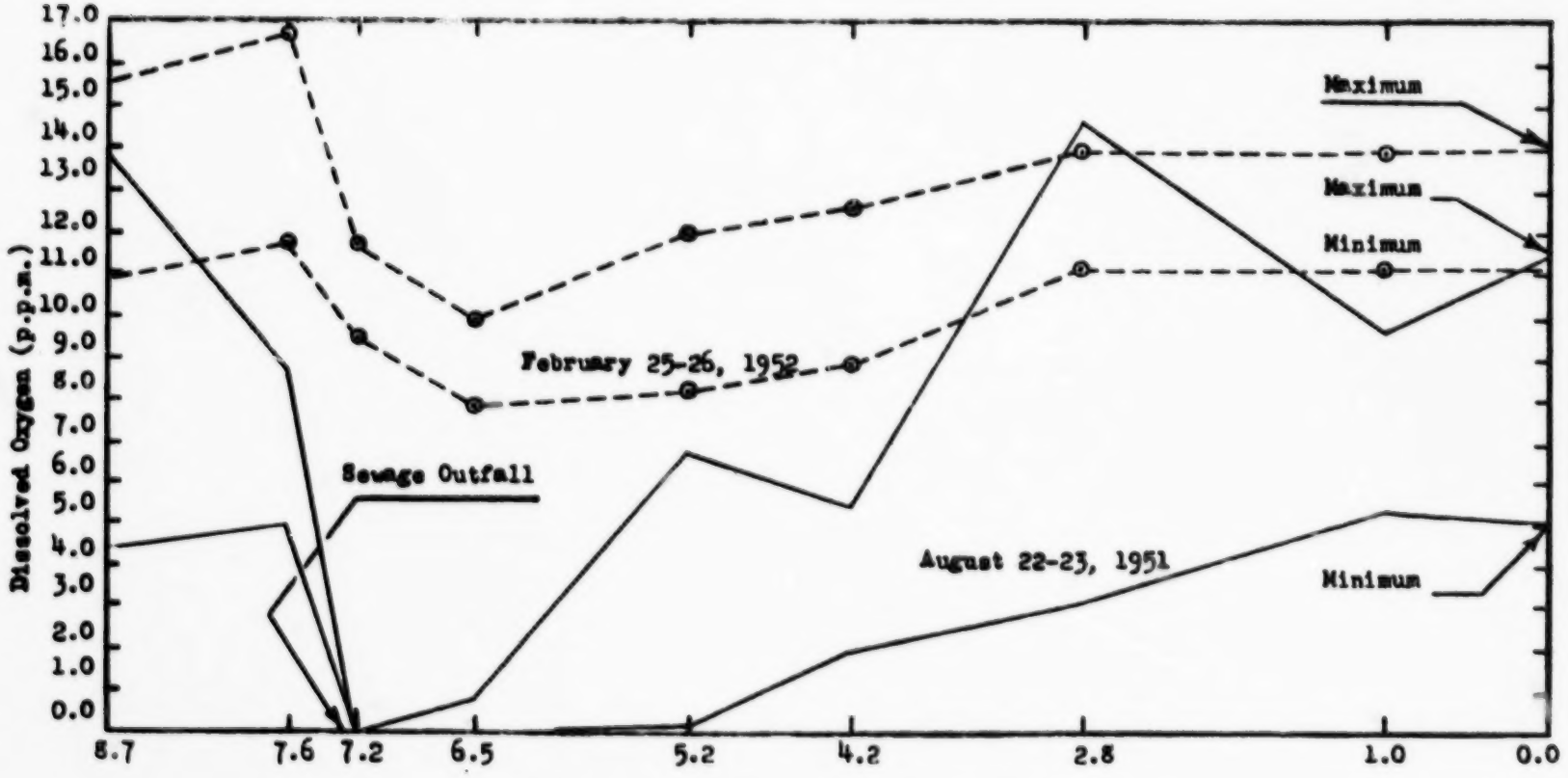


Figure 3

7-a

During the late spring nocturnal-diurnal variations in dissolved oxygen were large. For the 24-hour period, April 24-25, (Fig. 1) there was a considerable range in dissolved oxygen at all the sampling stations but it remained above 5 p.p.m. Summer conditions (Fig. 2) were considerably different. Oxygen was absent in two miles of the stream most of the day and the greatest oxygen concentration occurred in the lower recovery zone. The variation in dissolved oxygen over a 24-hour period was greatest in the lower recovery zone, being about 11 p.p.m. In streams such as this where products of the mineralization of large amounts of organic material stimulates plankton growth, a supersaturation of oxygen due to photosynthesis is an indicator of pollution.

Summer and winter conditions were compared in Figure 3. In winter, although there was a sag in the dissolved oxygen below the sewage outfall, there was an abundance of oxygen throughout the stream and daily cyclic variations in dissolved oxygen were much less than in summer. This reaffirms the fact that the season and time of day must be considered when samples are taken for the determination of dissolved oxygen deficiencies. Further, the highest oxygen levels usually occur between 2 and 4 o'clock in the afternoon, the lowest levels between 2 and 6 o'clock in the morning, and dissolved oxygen is usually not a problem during the winter months.

Studies of the bottom fauna and the fish of Lytle Creek have been carried on in conjunction with the physical chemical investigations. The aquatic life found in the various areas of the stream have been correlated with the environmental conditions which prevail. In polluted streams the composition

of resident populations varies greatly in different portions of the stream. Proceeding downstream from the point of pollution there is a gradual and marked change in the qualitative and quantitative composition of the flora and fauna. These differences were recognized during the last century and were so evident that several life zones were proposed and described for polluted streams. These zones are commonly designated as, degradation, septic, recovery, and clean water zones. The composition of an aquatic population in a given area is determined by environmental conditions, especially the extremes of these conditions. If any environmental factor reaches lethal levels at any time during the life history of a particular organism, that organism will disappear from the population even though the unfavorable conditions may be of short duration. This is well illustrated by temperatures and trout. If stream temperatures become too warm for trout during only one day a year, that stream does not support a native trout population. Because the qualitative and quantitative composition of aquatic populations are determined by the extremes of environmental factors, these populations serve to indicate habitat conditions which have prevailed during their life. Thus, one who knows the habitat requirements of aquatic organisms, especially non-migratory forms having an annual cycle, can, through a study of aquatic populations, judge quite accurately the worst conditions which have occurred in a stream during the past several months or during the life cycle of the organisms present. It is this indication of past ecological conditions which makes aquatic populations such valuable measures of pollutional conditions. Populations so used are designated as biological

indicators of pollution.

Single species or genera of tolerant forms are poor biological indicators. Tubifex or sludge worms are generally found in areas of gross organic pollution and are often spoken of as pollutional forms. They occur, however, in clean water areas and have been found in trout streams. The so-called pollutional or tolerant forms can live in clean waters but they become more numerous in polluted areas because food is abundant there and many of their predators and competitors are absent. In general, the absence of clean water forms which cannot withstand pollutional conditions are more indicative of pollution than is the abundance of tolerant forms. It is the composition of the aquatic population and the relative abundance of the different forms which is important in judging pollutional conditions. Populations and not individual species serve as biological indicators.

Life Characteristic of the Pollutional Zones

Organisms occurring in the septic zone are those which can survive at very low oxygen levels, as the tubificidae, those which can secure their oxygen from sources such as NO_3 and SO_4, and those which have adaptations for breathing atmospheric oxygen such as rat-tail maggots, mosquito larvae, and certain pulmonate snails. In the septic zone there are relatively few species of organisms, but there are a great many individuals of those species. In the clean water areas there are many more different species but individuals of these species occur in much smaller numbers (Fig. 4). Only 10 species were found in the septic zone of Lytle Creek but 70 species were taken in the clean water areas. In the septic zone the number of

Macro-Invertebrate Distribution, Lytle Creek - Summer Conditions

No. species ———; Volume organisms in cc.—·—·—; No. individual organisms, in thousands– – – –

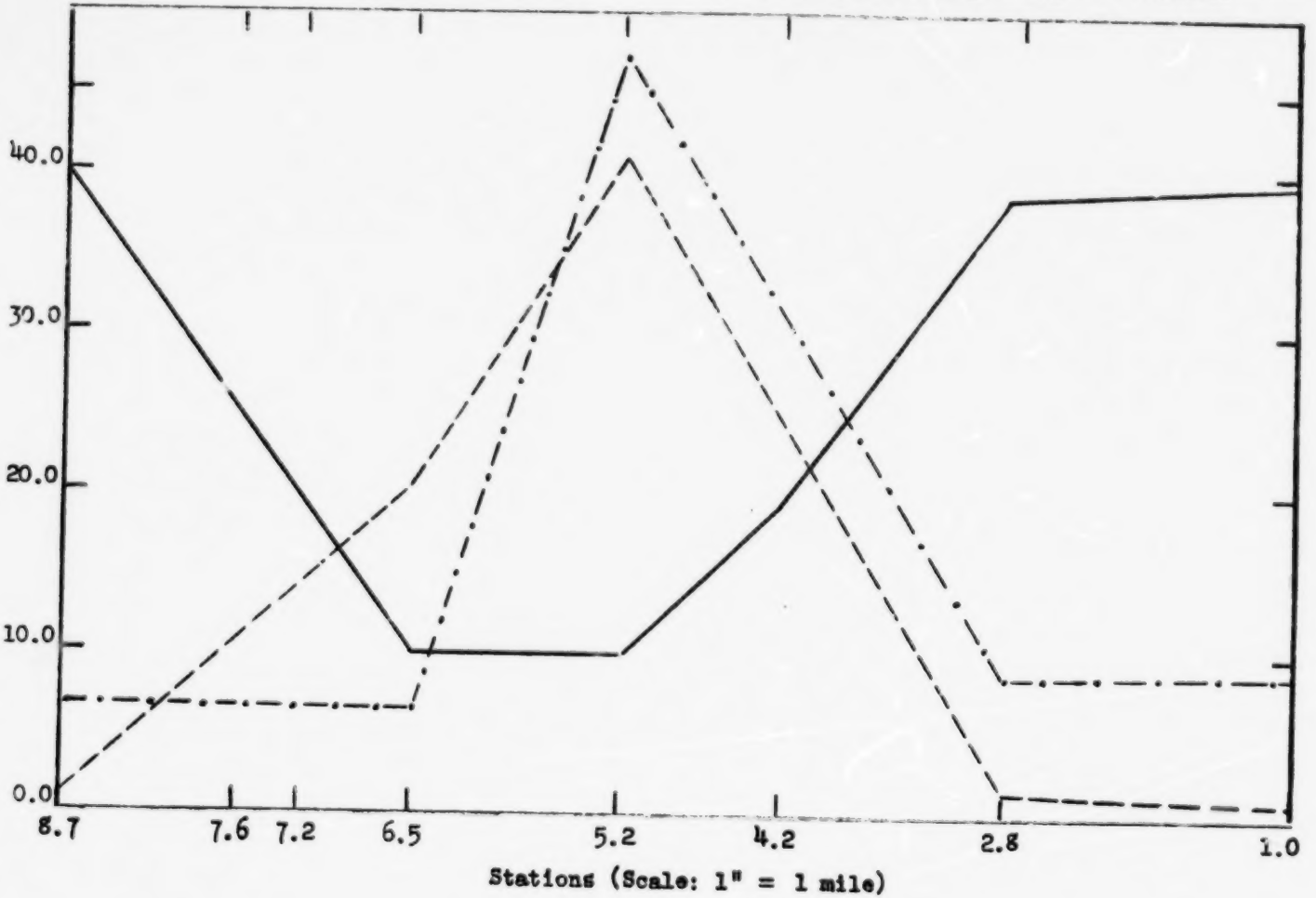


Figure 4

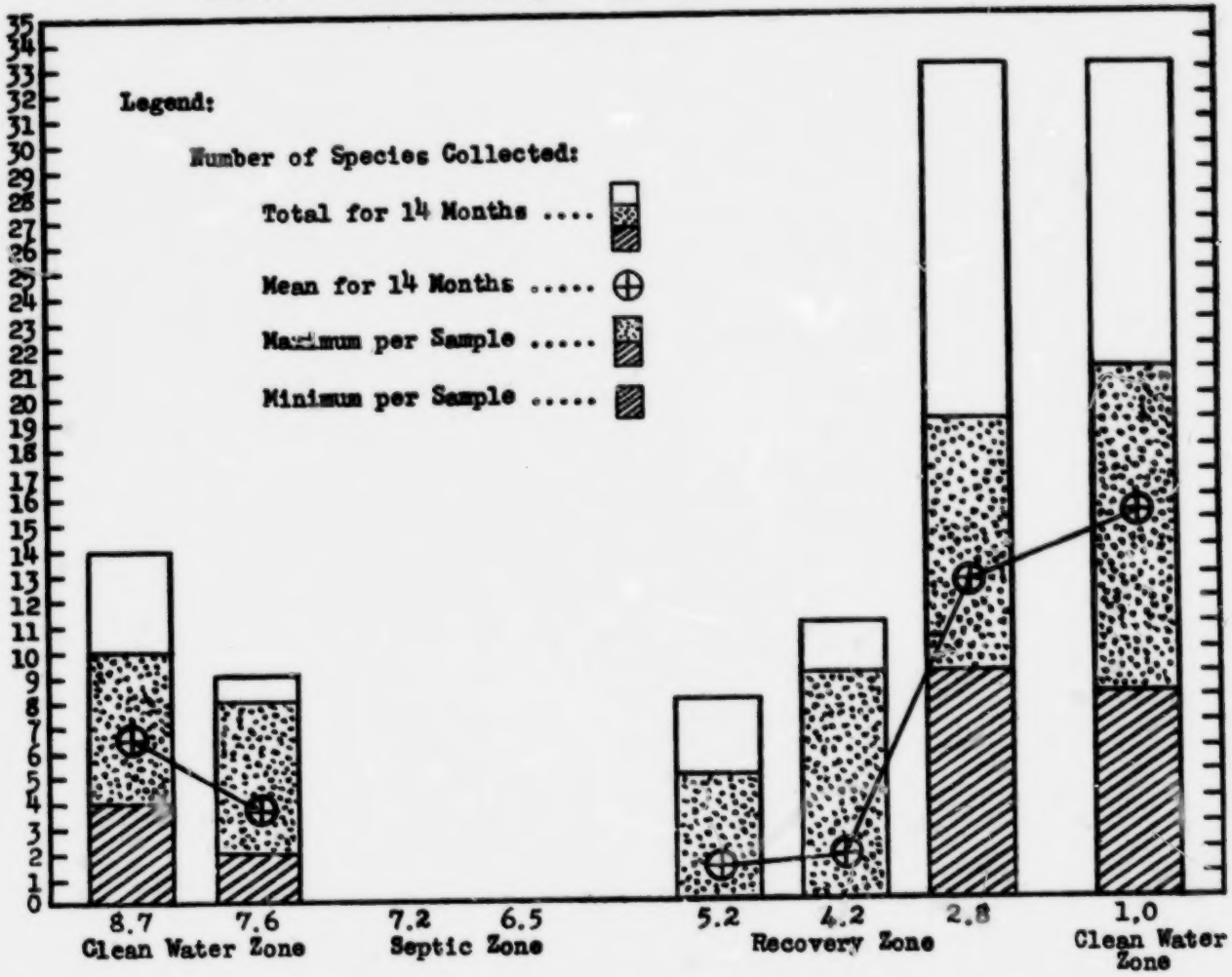
10-a

organisms taken per unit area of stream bottom was many times that found in the clean areas. Moving from the septic zone through the recovery zone, there was an increase in the numbers of different species and a great decrease in the number of individuals. The volume of organisms produced per unit of area was also many times greater in the septic zone than it was in the clean water zone. Organic pollutants exert a fertilizing effect on a stream and cause a greater production of aquatic life. If organic wastes are correctly treated and handled so that harmful effects are largely eliminated they can actually be used to increase fish yield. The great fish yields in the Illinois River in the first quarter of the century were largely due to the fertilizing effect of Chicago sewage.

Fish populations in the various pollutional zones follow, in part, the pattern of bottom organisms. There were, however, no fish in the septic zone. Tolerant forms were found in the recovery zone and the number of species and individuals increased with distance from the source of pollution. The number of species of fish taken over a 14 month period in various portions of Lytle Creek are shown in Figure 5. Large fish populations were found in the lower recovery and clean water zone. Even though there was abundant oxygen throughout the stream in winter, fish were not taken in those areas which were septic in summer. This may be due in part to the fact that the species of fish present in Lytle Creek are not very migratory. However, factors other than dissolved oxygen limit fish distribution in the creek. During the winter months fish were actually forced farther downstream than they were in summer. Apparently this was due to the

Lytle Creek

Effect of Domestic Sewage on the Number of Species of Fish



Stations in Miles Above Mouth of Stream

Figure 5

11-a

Reproduced from best available copy.

downstream extension of the pollutional blanket of bottom organisms. During winter, decreased temperatures slow down the activity of those bacteria which break down the organic materials and increased flows decrease the time of flow in the stream so that organic materials are carried farther downstream in winter. The pollutional blanket of bottom organisms, composed of bacteria (chiefly Sphaerotilus), protozoa, fungi, and organic debris which is characteristic of the septic zone in summer, also extends farther downstream, crowding out the bottom organisms which are important as fish food. This downstream movement results in a great decrease from October to January in the number of species of bottom organisms found in the lower portion of Lytle Creek (Fig. 6).

In summary, stream sanitation studies have indicated that: (1) there are great seasonal and nocturnal-diurnal environmental changes in polluted streams; (2) streams polluted with organic wastes have a definite succession of environmental conditions downstream from the point of waste discharge; (3) distinctive aquatic populations become established in these environmental zones; and (4) when the habitat requirements of these populations are understood they can be used to indicate past pollutional conditions in a stream.

Toxicity Studies

Investigations are being carried out on the toxicity of industrial wastes to fishes. At present these studies fall roughly under four headings: Literature survey, development of bio-assay methods, standardization of bio-assay methods, and toxicity tests of various ions, compounds and

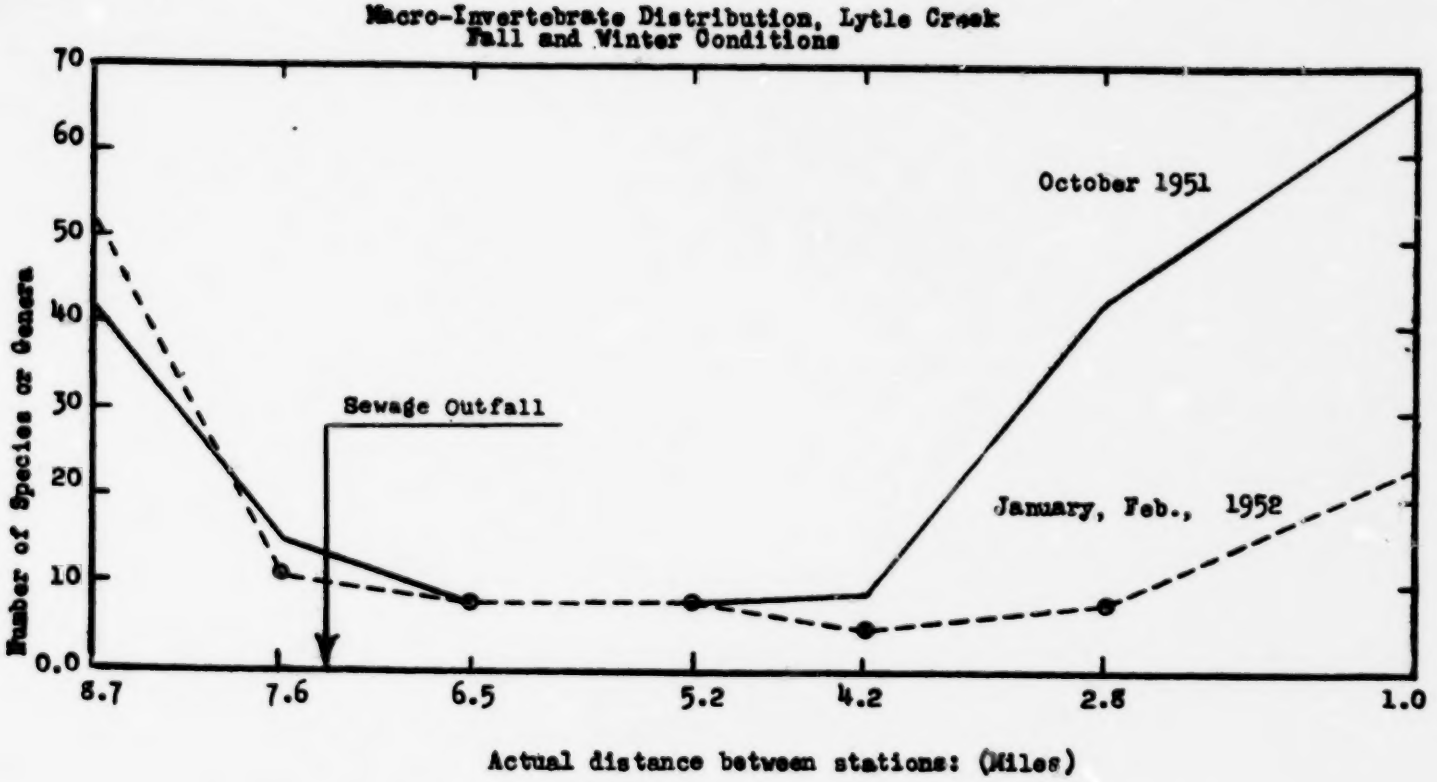

Macro-Invertebrate Distribution, Lytle Creek
Fall and Winter Conditions
Number of Species or Genera
70
60
50
40
30
20
10
0.0
October 1951
January, Feb., 1952
Sewage Outfall
8.7
7.6
6.5
5.2
4.2
2.8
1.0
Actual distance between stations: (Miles)

Figure 6

12-a

their mixtures.

A survey and critical evaluation is being made of the literature, dealing with the toxicity of industrial wastes to fishes. Insofar as possible a world-wide coverage is being made of articles appearing since 1910. The results of this survey are being reported in five parts each of which deals with a specific group of wastes. These groups of wastes are as follows: (1) alkalies, acids, and inorganic gases; (2) the metals as salts; (3) cyanides and miscellaneous inorganic substances; (4) organic compounds; and (5) complex industrial wastes. The evaluation of the first group appeared in the November, 1950, issue of the Journal of Sewage and Industrial Wastes. The second section on the metals as salts is now completed and will appear soon.

This survey and evaluation serves to summarize the information on the toxicity of industrial wastes to fishes and to indicate deficiencies in our knowledge and needs for further research. It is evident that more information is needed concerning the toxicity of various substances and mixtures of substances under a variety of conditions. This basic knowledge can be obtained through bio-assays of these materials using fish as reagents.

Bioassays of Toxic Wastes

Peter Doudoroff, In Charge
Toxicity and Fisheries Unit

Importance of direct approach to toxicity problems

Indirect methods of estimation of the toxicity of complex industrial wastes to aquatic life in the receiving waters, based on chemical assay of the concentrations of waste components known to be toxic, are too often unreliable. Because of synergy or antagonism, the mixtures can be much more toxic, or less toxic, than simple solutions of the individual substances involved. The dissolved minerals naturally present in highly variable amounts in receiving waters likewise can have a very pronounced influence upon the toxicity of a pollutant. Experimental toxicological data to be found in the literature have to do chiefly with the toxicity of solutions of some of the better known waste components considered individually. They do not show how much more toxic, or less toxic, these substances may be when present in all possible combinations, and under all possible conditions; and there is no reliable information about the toxicity of many of the more obscure industrial waste components which may be very important. Furthermore, chemical analytical procedures employed for the routine examination of industrial wastes are not always suitable for separating and identifying all the different components of complex wastes and measuring low concentrations of these chemicals. Substances which are very different toxicologically

often respond alike to the chemical tests employed.

Because of the unreliability of indirect methods, direct evaluation of industrial waste toxicity, through biological assay, involving the use of living organisms as reagents, often is necessary, in addition to any chemical examination undertaken.

Chemical criteria of water and effluent quality, in relation to aquatic life

A number of authors and regulatory agencies have attempted to define water pollution which is harmful to aquatic life in simple chemical terms. For reasons indicated above, such water quality criteria are necessarily based on incomplete data, and they can be very misleading. Synergetic and antagonistic interactions of toxicants and other dissolved substances are given little or no consideration. When limits of concentration of specific compounds are prescribed, the fact that the compounds may not be recognizable and may yield common toxic ions in mixed solutions is disregarded. When, on the other hand, only individual ions, elements and radicals are considered, the fact that their different combinations can have very different physiological effects is overlooked. Satisfactory water quality must be defined, therefore, in biological as well as in chemical terms.

Application of Toxicity Bio-assay Methods

Uniformity and reliability of toxicity bio-assay methods are essential for successful application of bio-assay results in the control of waste disposal. There can be no general agreement regarding the practical significance of these results, without some standardization of the

experimental procedures. Such standardization of method is the chief function of the committee established for that purpose within the Federation of Sewage and Industrial Wastes Associations. Much pertinent research also is needed, however, as a basis for increasingly sound interpretation and application of the data derived from the recommended routine toxicity tests.

Interactions of some toxic waste components as demonstrated by current research

Synergy of salts of heavy metals is exemplified by the extreme toxicity to minnows of mixed solutions of cupric and zinc salts in soft water (total alkalinity 17.5 p.p.m.). Only 0.025 p.p.m. of copper together with 1.0 p.p.m. of zinc killed most of the fish within 8 hours; whereas 0.2 p.p.m. of copper alone, and 8.0 p.p.m. of zinc alone were tolerated for 8 hours by most of the test animals under the same experimental conditions.

The detoxification of cyanide and of some toxic heavy metals through complexation (formation of metal-cyanide complexes) is now being intensively investigated. Ferrocyanides have long been known to be relatively non-toxic to fish, but the complexation of cyanide and ferrous ions in very dilute solutions has been found to be negligible in soft, slightly alkaline water. Furthermore, it has been shown by other investigators that ferrocyanides can be largely decomposed, with the release of highly toxic, free cyanide, under the influence of direct sunlight.

Zinc-cyanide and cadmium-cyanide complexes were found to be highly toxic, evidently because of a high degree of dissociation of the complex ions in very dilute solutions, and synergetic action of the metals and cyanide.

Complexation of nickel and cyanide occurs readily in dilute mixed solutions of their salts, and high concentrations of cyanide can be rendered relatively non-toxic in this way. However, even very low concentrations of nickel-cyanide complex have proved lethal to minnows under some conditions after prolonged exposure. Relatively concentrated solutions, which are highly alkaline, can be less toxic than much more dilute solutions, when these are prepared with the same soft water. Factors influencing the toxicity of the complex are being investigated, and it is evident that the pH of the solutions is of major importance.

The amount of free cyanide that can be effectively detoxified by complexation with copper was found to be limited, but low concentrations of cyanide and of extremely toxic cupric sulfate were rendered tolerable through the formation of a relatively stable complex. Under some conditions the detoxification of copper (which is difficult to remove completely from very dilute solutions) by the addition of cyanide is so effective that it may have some practical value in waste disposal.

The wastes from various metal-finishing plants usually are complex mixtures of different metal salts, cyanides, metal-cyanide complexes, and other chemicals. It is evident that the interactions of these components, which have received little attention in the past, are extremely important

and cannot be disregarded in seeking solutions of the difficult waste-disposal and waste-treatment problems of the metal-finishing industry. Waste-disposal practices and regulations devised without regard to these interactions are likely to be dangerous and fundamentally unsound.

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